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8. Alberg AJ, Korte JE. Invited commentary: parental smoking as a risk factor for adult tobacco use: can maternal smoking during pregnancy be distinguished from the social environmental influence during childhood? Am J Epidemiol 2014;179:1418-21.
9. Mannino DM, Caraballo R, Benowitz N, Repace J. Predictors of cotinine levels in US children: data from the Third National Health and Nutrition Examination Survey. Chest 2001;120:718-24.

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Comparing safety of abrasion and tape-stripping as skin preparation in allergen-specific epicutaneous immunotherapy

To the Editor:

Development of cutaneous delivery systems enabling needle-free and self-administrable vaccine administration is one important approach to improve global health care.

Passing the epidermal barrier structures is a challenge to the successful delivery of antigens: (1) the *stratum corneum*, composed of cornified keratinocytes surrounded by lipids, and (2) tight junctions in the *stratum granulosum*, providing ion and size selectivity.[1] Langerhans cells (LCs), able to capture antigen and induce adaptive immune responses, are found below these 2 barrier structures.[1] For efficient induction of immune responses, LCs need to be activated to elongate their dendrites through the tight junctions. Whether physical epidermal barrier disruption is necessary for efficient antigen capture is still a matter of debate.

Recently, a transcutaneous vaccine delivery platform was developed by Intercell and approved for prophylactic vaccination against Japanese encephalitis. For efficient immunization, an abrasive skin preparation system is used before vaccine delivery, based on trials showing direct correlation between degree of skin disruption and magnitude of antibody responses.[2,3] Besides facilitating skin permeability, mechanical barrier-disruption also activates and polarizes LCs through the release of cytokines acting as adjuvant.[4]

We recently demonstrated the efficacy of *epicutaneous* immunization in allergy immunotherapy[5,6] using adhesive tape-stripping for skin preparation and found higher allergen doses showing higher clinical efficacy.[5] We therefore aimed to enhance allergen delivery by using abrasion rather than tape-stripping for skin preparation as shown for other transcutaneous vaccines.[3] However, enhanced skin disruption may represent a safety risk in allergic individuals.

A detailed methodology is given in this article's Online Repository at www.jacionline.org. Briefly, a total of 98 grass-pollen–allergic patients were included and randomly assigned to receive placebo epicutaneous allergen-specific immunotherapy (EPIT) (1.5 mL petrolatum) or allergen EPIT (1.5 mL allergen-extract, Stallergènes, 200 IR/mL). Before application of the first patch, 2 skin-preparation procedures were tested: abrasion with a foot-file (52 patients: 26 placebo and 26 allergen) or adhesive tape-stripping (45 patients: 21 placebo and 24 allergen) (see Fig E1 in this article's Online Repository at www.jacionline.org). The degree of skin disruption was measured by transepidermal water loss (TEWL) before and after skin preparation. For safety, patients were observed for 30 minutes and local immediate-type reactions such as erythema and pruritus were assessed 10 and 30 minutes after patch application. Pruritus intensity was rated on a visual analog scale from 0 to 100. The patch was removed after 8 hours. Delayed-type reactions, such as eczema, were monitored by phone call 48 hours after patch application.

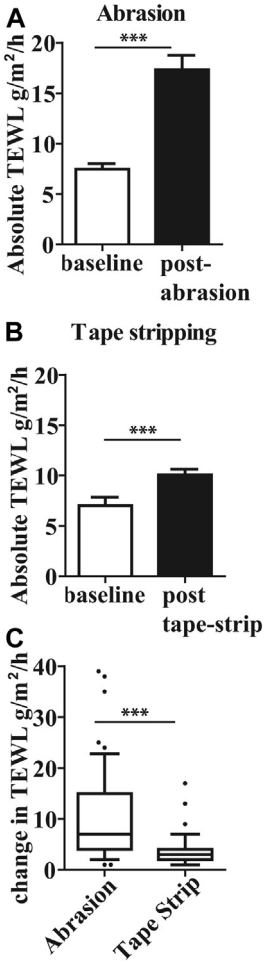

FIG 1. Absolute TEWL before and after skin pretreatment with either abrasion **(A)** or tape-stripping **(B)**. Data are shown as mean ± SEM, comparison by Wilcoxon signed rank test. **C,** Comparison of change in TEWL after abrasion and tape-stripping by using the Mann-Whitney test. Box plots indicate the median, the 10th, 25th, 75th, and 90th percentiles, and outliers. ***$P < .001$.

Both skin-preparation methods, abrasion (Fig 1, *A*) and tape-stripping (Fig 1, *B*), resulted in significantly increased TEWL when compared with baseline ($P < .0001$). The mean change in TEWL after abrasion, 10.20 g/m^2/h (median, 7 g/m^2/h; 95% CI, 7.64-12.75), was significantly higher than after tape-stripping, 3.71 g/m^2/h (median, 3 g/m^2/h; 95% CI, 2.74-4.67) (Fig 1, *C*; $P < .0001$). Correspondingly, local immediate-type reactions such as pruritus appeared faster and significantly stronger after allergen EPIT with abrasion as compared with allergen EPIT with tape-stripping (Fig 2, *A* and *B*). Indeed, a strong correlation was observed between change in TEWL and pruritus intensity (Fig 2, *D* and *E*; $P = .0015$). Again, erythema (Fig 2, *C*) was significantly stronger after allergen EPIT with abrasion (mean erythema size, 58.44 ± 32.36 cm^2) than after allergen EPIT with tape-stripping (6.25 ± 19.32 cm^2) and significantly more frequent ($P < .001$). Local eczema was reported only after allergen EPIT: 7

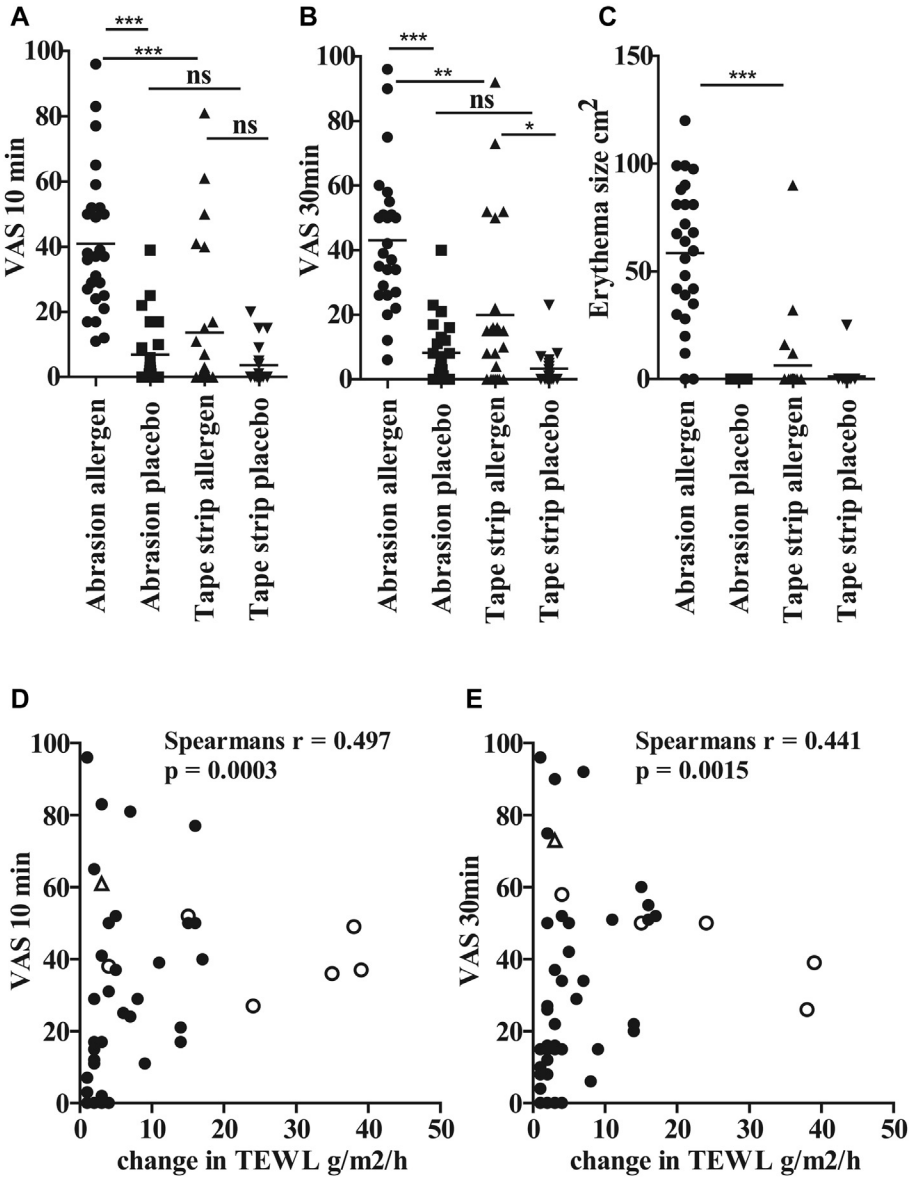

FIG 2. Local itching intensity recorded by VAS 10 minutes **(A)** and 30 minutes **(B)** after different EPIT pretreatments. **C,** Erythema size 30 minutes after treatment. Group comparison by using the Kruskal-Wallis test. ***$P < .001$, **$P < .01$, and *$P < .05$. Correlation between itching intensity by VAS and change in TEWL 10 minutes **(D)** and 30 minutes **(E)** after allergen EPIT. Patients with systemic allergic reaction after abrasion are depicted as open circles and after tape-stripping are depicted as triangles. (One patient suffering from a systemic allergic reaction after abrasion showing TEWL change of 35 g/m^2/h is omitted in Fig 2, *E*, because the corresponding VAS value is missing.) *ns*, Nonsignificant; *VAS*, visual analog scale.

patients (26.9%) after abrasion and 4 patients (16.6%) after tape-stripping ($P = .41$).

Systemic allergic reactions were observed in 6 patients after allergen EPIT with abrasion and in 1 patient after allergen EPIT with tape-stripping ($P = .10$; see Table E1 in this article's Online Repository at www.jacionline.org). When pooling results of our previous trials,[5,6] systemic reaction frequency after abrasion was significantly higher than after tape-stripping ($P = .033$; see Table E2 in this article's Online Repository at www.jacionline.org). All patients with a change in TEWL higher than 20 g/m^2/h experienced a systemic allergic reaction (Fig 2, *D* and *E*).

Concluding on our study, stronger skin-barrier disruption after abrasion than after tape-stripping was associated with significantly stronger local pruritus and erythema size, and a more systemic allergic reaction, indicating that safety is likely more critical in allergen EPIT than for other vaccines. As a comparison, skin preparation by abrasion using the skin preparation system before transcutaneous vaccination for *E coli*–induced traveller's diarrhea was completely safe.[3] Reported average change in TEWL was 12.9 g/m^2/h and even vaccines with changes in TEWL between 20 g/m^2/h and 70 g/m^2/h did not show any systemic adverse effects,[3] whereas in allergen EPIT all patients with changes in TEWL above 20 g/m^2/h suffered from systemic allergic reactions. Hence, although strong skin-barrier disruption is unproblematic in transcutaneous prophylactic vaccination, it seems dangerous in allergen EPIT, where

patients are allergic to the delivered protein. Hence, if a large amount of allergen penetrates beyond the nonvascularized epidermis toward the dermal vasculature, systemic allergic reactions are frequent, as observed in 6 patients after abrasion. In contrast, only 1 patient developed a systemic allergic reaction after tape-stripping, which has been shown to primarily affect the *stratum corneum*, with allergen deposition in epidermal layers.[7] Eczema was reported after allergen EPIT with abrasion and with tape-stripping but in no patient after placebo EPIT. Please note patient self-reporting of eczema as a weakness of this study.

The frequency of adverse effects after allergen EPIT with tape-stripping is comparable to our previous experience[5,6] (see Table E2) except for 2 striking differences: (1) in the first study with 48-hour patch administration time, more eczema reactions were reported, and (2) in the present subanalysis, the number of systemic adverse effects was more than doubled when using abrasion than when using tape-stripping.

As a limitation, we could not show statistical significance for difference in systemic allergic reaction frequency after allergen EPIT with abrasion and tape-stripping when strictly using data of the present subanalysis of ClinicalTrials.gov NCT00777374. Because end points were efficacy and immunological changes, no power calculations were done for the present retrospective analysis. As another limitation, skin abrasion using a foot-file is difficult to standardize and the procedure was performed by different study team members. Furthermore, because abrasion had to be stopped prematurely for safety reasons, no analysis with respect to enhanced efficacy could be performed.

Also, it would be interesting to measure the modulation of immune responses after abrasion versus tape-stripping because the degree of skin disruption has been suggested to play a role in polarizing T_H1, T_H2, or T-regulatory-cell–type responses by activating different subsets of skin-resident antigen-presenting cells.[8] Skin-barrier disruption has been reported to favor T_H2-polarized immune responses,[1] while hydration-facilitated antigen delivery on nondisrupted skin favors T-regulatory-cell responses.[9] Hence, skin-barrier disruption would seem disadvantageous for allergen immunotherapy. Nevertheless, we previously showed the therapeutic efficacy of allergen EPIT with tape-stripping.[5,6] Therefore, the effects of different methods for skin disruption on T-cell polarization are so far unclear.

Here, we highlight that different skin-disruption methods must also be compared for safety, adding another layer of complexity in the field of allergy immunotherapy.

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REFERENCES

1. De Benedetto A, Kubo A, Beck LA. Skin barrier disruption: a requirement for allergen sensitization? J Invest Dermatol 2012;132:949-63.
2. Frerichs DM, Ellingsworth LR, Frech SA, Flyer DC, Villar CP, Yu J, et al. Controlled, single-step, stratum corneum disruption as a pretreatment for immunization via a patch. Vaccine 2008;26:2782-7.
3. Glenn GM, Villar CP, Flyer DC, Bourgeois AL, McKenzie R, Lavker RM, et al. Safety and immunogenicity of an enterotoxigenic *Escherichia coli* vaccine patch containing heat-labile toxin: use of skin pretreatment to disrupt the stratum corneum. Infect Immun 2007;75:2163-70.
4. Nickoloff BJ, Naidu Y. Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin. J Am Acad Dermatol 1994;30:535-46.
5. Senti G, von Moos S, Tay F, Graf N, Sonderegger T, Johansen P, et al. Epicutaneous allergen-specific immunotherapy ameliorates grass pollen-induced rhinoconjunctivitis: a double-blind, placebo-controlled dose escalation study. J Allergy Clin Immunol 2012;129:128-35.
6. Senti G, Graf N, Haug S, Ruedi N, von Moos S, Sonderegger T, et al. Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. J Allergy Clin Immunol 2009;124:997-1002.
7. Chen X, Shah D, Kositratna G, Manstein D, Anderson RR, Wu MX. Facilitation of transcutaneous drug delivery and vaccine immunization by a safe laser technology. J Control Release 2012;159:43-51.
8. Merad M, Ginhoux F, Collin M. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. Nat Rev Immunol 2008;8:935-47.
9. Dioszeghy V, Mondoulet L, Dhelft V, Ligouis M, Puteaux E, Benhamou PH, et al. Epicutaneous immunotherapy results in rapid allergen uptake by dendritic cells through intact skin and downregulates the allergen-specific response in sensitized mice. J Immunol 2011;186:5629-37.

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IgE antibodies to mountain cedar pollen predominantly recognize multiple conformational epitopes on Jun a 1

To the Editor:

Recent analyses of several databases of allergens provide evidence for structural similarities of allergenic proteins.[1] The 707 allergens with known sequences belong to only 184 (~2%) of the 9318 protein families (Pfams).[2] Furthermore, of the rare Pfams that contain an allergen, 81 contain multiple allergens and 10 Pfams with the most allergens contain 300 (42%) allergens. The congruence of pollen allergens with structural families is even more apparent. Of the 157 pollen allergens with known sequences, 93 (59%) reside in just 5 Pfams,[3] suggesting that pollen allergens share a very limited number of relatively unique structures. Identifying the common structural features of allergens may help to elucidate their unique structural elements and potentially the mechanism(s) for their allergenicity.

The goal of our research was to use the highly allergenic mountain cedar (*Juniperus ashei*, Cupressaceae) pollen as a model for characterizing the allergenicity of individual proteins and to identify the structural elements that are required for allergic sensitization or reactions.[4] In the current study, we first quantified patient IgE antibodies to crude extract of cedar pollen and to purified Jun a 1,[5] a major mountain cedar allergen, using ImmunoCap technology (Phadia, Uppsala, Sweden). The vast majority (median, 93%) of IgE antibodies to cedar pollen in the serum from 35 allergic subjects (34 subjects; see Table E2 in this article's Online Repository at www.jacionline.org) reacted with Jun a 1 (see Fig E1 in this article's Online Repository at www.jacionline.org).

To define the fine specificity and complexity of these antibodies, we chose 7 sera with high concentrations of IgE anti–Jun a 1 antibodies and adequate serum volume to assess the relative

METHODS

Clinical trial design and participants

This study was a retrospective analysis of a single-center phase I/IIa, placebo controlled, randomized, double-blind study conducted at the Clinical Trials Center of the University Hospital of Zurich, Switzerland (ClinicalTrials.gov NCT00777374). The study was designed to test safety and clinical efficacy of EPIT. Two different skin preparation procedures were tested before application of the first EPIT patch: adhesive tape-stripping and abrasion using a foot-file.

A total of 98 patients were enrolled. Inclusion criteria were signed informed consent, age between 18 and 65 years, and a history of grass-pollen–allergic rhinoconjunctivitis with positive skin prick and conjunctival provocation test results. Patients with eczematous skin lesions on the upper arms, perennial allergic rhinitis, moderate to severe asthma, mastocytosis, malignancy, active infectious disease, or significant systemic illness or if pregnant or nursing were excluded. Those using the following drugs were excluded from study participation: antihistamines with a long half-life within the last week, systemic or topical steroids within the last 5 days, beta-blockers, immunosuppressive agents, angiotensin-converting enzyme inhibitors, angiotensin II antagonists, steroid inhalers, or tricyclic antidepressants.

Study procedures and skin pretreatment methods

The first of the 6 patches was administered in January 2009, that is, approximately 3 months before the start of the grass pollen season. The skin on the upper arm was prepared by either adhesive tape-stripping or abrasion and the patch was then applied for 8 hours. Adhesive tape-stripping was performed 10 times on an area of 16 cm^2 using a scotch tape (Scotch Magic Tape 810, 3M Company, St Paul, Minn). A new tape was used for each of the 10 strips. Abrasion was performed with a commercially available foot file (Pedic Care, Migros, Switzerland) with a 100 grit size. The file was applied once tangentially by trained study personnel.

Patch and test drug

The patch system was provided by Medanz Medical GmbH (Starnberg, Germany). The allergen extract of 5 grasses (*Dactylis glomerata*, *Agrostis stolonifera*, *Phleum pratense*, *Poa pratensis*, and *Anthoxanthum odoratum*) was purchased from Stallergènes (Antony, France) with a biological activity of 200 IR/mL suspended in white petrolatum. Individual patches were manufactured by the cantonal pharmacy of Zurich and loaded with 1.5 mL of allergen extract or with placebo (white petrolatum only). This allergen concentration and formulation was already used in our first trial.[E1]

TEWL measurement

Measurement of TEWL is a common method used for the assessment of *stratum corenum* barrier function.[E2] The TEWL was measured with the DermaLab USB device (Hadsund, Denmark) before and after skin pretreatment as described.[E3] The patients had 15 minutes acclimatization to room temperature before the TEWL assessment. The probe was held with isolating latex gloves and then placed onto the untreated skin area for acquisition of baseline TEWL. Thereafter, the skin was treated either by tape-stripping or by abrasion, and the TEWL was measured again. The TEWL values were noted once steady state had been reached. Change in the TEWL was calculated by subtraction of the individual baseline value from the post-treatment value.

Safety

After patch application, patients were supervised for 30 minutes in the clinical trials facility. If no systemic events were observed, patients were discharged with an emergency set containing the antihistamine levocetirizine 20 mg (UCB Phama, Bulle, Switzerland) as well as prednisone 100 mg (Streuli, Uznach, Switzerland).

Local itching at the patch application site was assessed 10 and 30 minutes after patch application using a visual analogue scale ranging from 0 to 100. At the same time, erythema size was measured. Forty-eight hours after patch application, patients were contacted by phone to assess local late-type eczematous skin reaction. Local eczematous skin reactions were graded according to the criteria of atopy patch test after the revised European Task Force on atopic dermatitis key for atopy patch test reading[E4]: "−" negative; "?" only erythema; "+" erythema, infiltration; "++" erythema, few papules; "+++" erythema, many or spreading papules; "++++" erythema, vesicles.

Systemic allergic reactions as a response to patch application were graded according to the EAACI Immunotherapy Task Force[E5]: Grade 0: nonspecific reactions such as discomfort, headache, and arthrlagia; grade 1: mild systemic reactions such as localized urticaria, rhinitis, or mild asthma (peak plow [PF] <20% decrease from baseline); grade 2: moderate systemic reactions such as slow onset (>15 minutes) of generalized urticaria and/or moderate asthma (PF <40% decrease from baseline); grade 3: severe (not life-threatening) systemic reactions with rapid onset (<15 minutes) of generalized urticaria, angioedema, or severe asthma (PF >40% decrease from baseline); grade 4: anaphylactic shock with immediately evoked reaction of itching, flushing, erythema, generalized urticaria, stridor (angioedema), immediate asthma, hypotension.

Statistical analysis

Because this study is retrospective analysis of a clinical trial determining the safety and efficacy of allergen EPIT by comparing it to placebo EPIT (ClinicalTrials.gov NCT00777374), sample size calculation was powered to show the clinical efficacy of allergen EPIT compared with that of placebo EPIT. No sample size was calculated for this subanalysis to show difference in systemic allergic reactions between different skin pretreatment groups.

Statistical analyses were performed using the Graph pad software. Gaussian distribution was tested using D'Agostino and Pearson normality test. Wilcoxon matched pairs test was used to compare values before and after skin pretreatment. For intergroup comparison of nonparametric data, 2-way Mann Whitney *U* test was applied. Multiple group comparison was done using the Kruskal Wallis test with Dunn's multiple comparison tests. Correlation was analyzed using the Spearman test. The frequency of systemic and local allergic reactions (erythema and eczema) after allergen EPIT occurring in the different pretreatment groups was compared using Fisher exact test with mid-*P* adjustment.

REFERENCES

E1. Senti G, Graf N, Haug S, Ruedi N, von Moos S, Sonderegger T, et al. Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. J Allergy Clin Immunol 2009;124:997-1002.
E2. Fluhr JW, Feingold KR, Elias PM. Transepidermal water loss reflects permeability barrier status: validation in human and rodent in vivo and ex vivo models. Exp Dermatol 2006;15:483-92.
E3. Pinnagoda J, Tupker RA, Agner T, Serup J. Guidelines for transepidermal water loss (TEWL) measurement: a report from the Standardization Group of the European Society of Contact Dermatitis. Contact Dermatitis 1990;22:164-78.
E4. Turjanmaa K, Darsow U, Niggemann B, Rance F, Vanto T, Werfel T. EAACI/GA2LEN position paper: present status of the atopy patch test. Allergy 2006;61:1377-84.
E5. Alvarez-Cuesta E, Bousquet J, Canonica GW, Durham SR, Malling HJ, Valovirta E. Standards for practical allergen-specific immunotherapy. Allergy 2006;61:1-20.
E6. Senti G, von Moos S, Tay F, Graf N, Sonderegger T, Johansen P, et al. Epicutaneous allergen-specific immunotherapy ameliorates grass pollen-induced rhinoconjunctivitis: a double-blind, placebo-controlled dose escalation study. J Allergy Clin Immunol 2012;129:128-35.

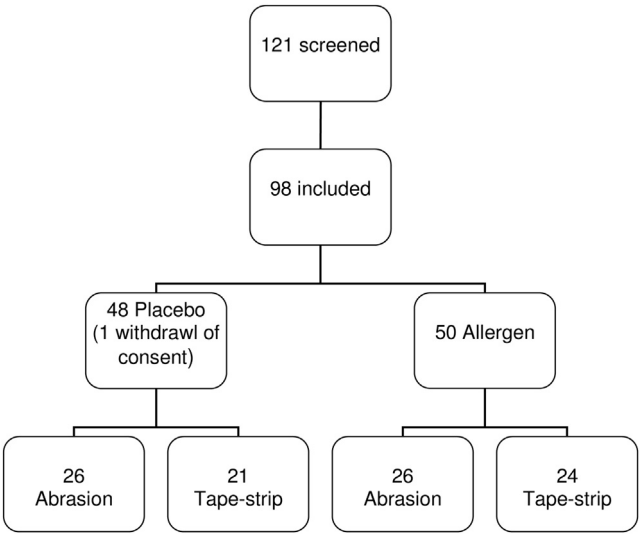

FIG E1. Participant flow. Flow diagram showing patient allocation to different study groups and different skin pretreatment procedures before the application of the first EPIT patch. One patient to receive placebo EPIT with tape-stripping declined further participation.

TABLE E1. Systemic allergic reactions

Systemic allergic reaction	Abrasion		Tape-stripping	
	Allergen (n = 26)	Placebo (n = 26)	Allergen (n = 24)	Placebo (n = 21)
Grade I	1	0	0	0
Grade II	5	1	1	0
Grade III	0	0	0	0
Grade IV	0	0	0	0
Total, n (%)	6 (23)	1 (3.8)	1 (4.2)	0

TABLE E2. Comparison of EPIT trials

Trial characteristic	First trial (pollen season 2006)[E1]	Second trial (pollen season 2007)[E6] NCT00719511	Third trial (pollen season 2009) NCT00777374
Allergen extract	5 Graminees, Stallergènes, France	6 Graminees, Inmunotek, Spain	5 Graminees, Stallergènes, France
Solvent	Petrolatum	Glycerol 50%	Petrolatum
Allergen extract potency	200 IR/mL, 1.5 mL = 21 μg Phl p 5	10 HEP/mL, 1 mL = 3 μg Phl p 5 50 HEP/mL, 1 mL = 15 μg Phl p 5 100 HEP/mL, 1 mL = 30 μg Phl p 5	200 IR/mL, 1.5 mL = 21 μg Phl p 5
Skin preparation	Tape-stripping 6×	Tape-stripping 6×	Tape-stripping 10× *Before first patch (subanalysis)* Tape-stripping/abrasion
Duration of patch application (h)	48	8	8
Number of patches	12	6	6
Safety: Eczema (eczema/total patch applications)	160/252 (63.5%)	10 HEP: 29/174 (16.6%) 50 HEP: 51/171 (29.8%) 100 HEP: 44/171 (25.7%)	48/265 (18.1%) in total *Before first patch (subanalysis)* Tape-stripping: 4/24 (16.6%) Abrasion: 7/26 (26.9%)
Safety: Systemic adverse effects/total patients	0/21 (0%)	10 HEP: 3/33 (9%) 50 HEP: 3/33 (9%) 100 HEP: 4/33 (12%)	Tape-stripping: 1/24 (4%) Abrasion: 6/26 (23%)
Total (pooled analysis) Number of patients with systemic adverse effects/number of patients without systemic reaction	Tape-stripping: 11/133 Abrasion: 6/20		